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From: Gambel, Phillip
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observed. In the absence of BIS20x3 cytotoxicity did not exceed 20% at all ET's. We have initiated experiments in which the role of several T-cell induced apoptotic pathways will be examined in the bsAb-setting.

#1830 PURGING OF TUMOR CELLS FROM CRYOPRESERVED OVARIAN TISSUE OF CANCER PATIENTS BY MEANS OF THE BIS-1 ANTIBODY. Hetty Timmer-Bosscha, Caroline P Schroder, Harrie Hollema, Lou F H M de Ley, Maas-Jan Heineman, and Elisabeth G E de Vries, *Univ Hosp, Groningen, Netherlands*

Introduction: Aggressive therapy for the treatment of cancer can lead to impaired fertility in female patients, but cryopreservation and autografting of ovarian tissue could be a method for conserving their fertility. However, tumor cell contamination of autografts may form a problem. Epithelial tumor cell lysis can be obtained by cytotoxic T cell retargeting with bispecific antibody BIS-1, which combines affinity for the T cell receptor and epithelial glycoprotein-2 (EGP-2). Aim: To examine in vitro purging of epithelial tumor cells from cryopreserved ovarian autografts, by means of BIS-1. Methods: Human ovarian tissue was thawed,washed in Leibovitz medium with a sequence of decreasing DMSO, and incubated in medium containing Dnase I and collagenase I. In a ratio of 1:100 upto 1:10000, tumor cells (EGP-2 positive breastcancer cell line MCF-7) labelled with an intracellular fluorescent dye and activated human lymphocytes were added. The suspension was incubated for 2 or 16 h in medium containing 0.1 μg/mL BIS-1. Finally, the viability of ovarian tissue and the presence of MCF-7 tumor cells was evaluated by means fluorescence microscopy or standard (immunocytochemical) staining protocols. Results: After 2h purging with a target/lymfocyte ratio of 1:10000, or 16 h with 1:100, no viable MCF-7 tumor cells could be identified. Ovarian tissue contained intact and viable follicles. Also, lymphocytes were present in the remaining cell suspension. Conclusions: These results show that purging of epithelial tumor cells from ovarian grafts by means of BIS-1 is possible in vitro. Intact and viable follicles can still be identified after this procedure. This method may contribute to the safe replacement of ovarian tissue in female cancer survivors.

#1831 PRODUCTION OF A SINGLE CHAIN VARIABLE FRAGMENT ANTIBODY RECOGNIZING TYPE III MUTANT EPIDERMAL GROWTH FACTOR RECEPTOR. Kazuhiro Yoshikawa, Norihisa Nakayashiki, Kazuyasu Nakamura, Nobuo Hanai, Syo Okamoto, Masaaki Mizuno, Toshihiko Wakabayashi, Shinsuke Saga, Jun Yoshida, and Toshitada Takahashi, *Aichi Cancer Center Research Institute, Nagoya, Japan, Aichi Medical University, Aichi, Japan, Kyowa Hakko Kogyo Co. Ltd., Machida, Japan, and Nagoya University, Nagoya, Japan*

The type III deletion mutant of the epidermal growth factor receptor (EGFR) is a potential target in diagnostic and therapeutic approaches for these glioblastomas characterized by its expression. We previously raised a mouse monoclonal antibody, 3C10 (IgG2b) specifically recognizing this mutant EGFR by immunization with the mutant peptide. In this study, a single chain variable fragment (scFv) antibody was produced. Partialy determinatain of its N-terminal amino acid sequence and preparation of adequate primers for variable heavy chain (VH) and variable light chain (VL) genes were performed to allow cloning of the VH and VL genes by the reverse transcriptase-polymerase chain reaction (RT-PCR). The genes cloned were assembled with a linker, (Gly4Ser)3 and ligated into a bacterial expression vector to express the scFv as cytoplasmic inclusion bodies. After appropriate refolding, the antibody activity of VH-VL scFv was examined in an exzyme-linked immunosorbent assay. 3C10 scFv showed a selective reactivity with the mutant peptide, similar to the parental 3C10 antibody. A mouse transfectant expressing type III mutant was positively stained by immuno fluorescence, as well as a glioblastoma specimen with the type III deletion.

IMMUNOLOGY/PRECLINICAL AND CLINICAL 9: Novel Antibodies and Fusion Proteins II

#1832 RITUXIMAB-MEDIATED GROWTH REGULATION AND DRUG SENSITIZATION OF B LYMPHOMA: PIVOTAL ROLE OF IL-10. Steve Alas, Christos Emmanouilides, Nabil Hanna, and Benjamin Bonavida, *IDEC Pharmaceuticals Corp, San Diego, CA, and Univ of CA, Los Angeles, Los Angeles, CA*

Treatment of human B tumor cells with Rituximab inhibits cell proliferation and sensitizes cells to various cytotoxic drugs. This study delineates a possible mechanism by which Rituximab induces its effects. We show that Rituximab inhibits the secretion of IL-10 in B lymphoma cells. Neutralization of IL-10 using polyclonal antibodies results in decreased proliferation in these B cells. Furthermore, exogenous IL-10 increases cell growth, even in the presence of Rituximab. This suggests that IL-10 acts as a growth factor in B cell lymphoma. The role of IL-10 was also examined in Rituximab-mediated drug sensitization of tumor B cells. Rituximab down-regulates expression of Bcl-2, but not of other cancer related genes. The addition of exogenous IL-10 during Rituximab treatment up-regulates Bcl-2 levels. These findings suggest that IL-10 acts as a protective factor, as well. We also demonstrate that IL-10 triggers IL-10 receptor to induce JAK phosphorylation of STAT3 in these lymphoma cells. We propose that endogenous IL-10 initiates the JAK/STAT pathway whereby STAT3 binds to one of two STAT3 binding sites upstream of the bcl-2 gene and initiates transcription. These IL-10 autocrine/paracrine loops may maintain a basal level of Bcl-2 within each cell, conferring resistance to chemotherapeutic drugs. We further propose that reduction of IL-10 in the tumor microenvironment by Rituximab may potentially diminish the level of constitutively activated STAT3 and concomitantly reduce expression of Bcl-2, serving as a mechanism to sensitize resistant tumor cells to low doses of drugs.

#1833 DEVELOPMENT OF PSMA-BASED IMMUNOTHERAPIES FOR PROSTATE CANCER. Gerald P Donovan, Donna M Morrissey, William C Olson, Warren D W Heston, and Robert J Israel, *Progenics Pharmaceuticals, Inc, Tarrytown, NY, PSMA Development Company, LLC, Tarrytown, NY, and The Cleveland Clin, Cleveland, OH*

Prostate-Specific Membrane Antigen (PSMA) is a type-2 membrane protein that is abundantly expressed in prostate cancer but not normal human tissues. An antibody to the intracellular portion of PSMA has been licensed by FDA for *in vivo* imaging of prostate cancer and provides clinical proof-of-concept for PSMA-based tumor targeting. The large extracellular portion of PSMA constitutes the majority of the molecule (707 of 750 amino acids) and provides an attractive target for therapeutic monoclonal antibodies and vaccines. We have generated murine cell lines that express high levels of full-length human PSMA and immunized mice with these cells for the purposes of making a panel of monoclonal antibodies to epitopes scattered throughout the native extracellular domain of PSMA. We have also created recombinant cell lines that mediate high level secretion of truncated forms of PSMA for evaluation as vaccines in combination with experimental adjuvants. Lastly we have constructed nonreplicating viral vectors that encode membrane-bound and secreted forms of PSMA as a potential means of eliciting robust cellular and humoral immune responses to selected regions of the protein. These studies are designed to optimize the utilization of PSMA for active and passive immunotherapies of prostate cancer.

#1834 CYTOTOXICITY OF AN ANTI-CD22 RECOMBINANT IMMUNOTOXIN (BL22) AGAINST B-CELL PRECURSOR ACUTE LYMPH BLASTIC LEUKEMIA (BCP-ALL) CELLS FROM PEDIATRIC PATIENTS. Glen Lew, Muxiang Zhou, Lubing Gu, David J FitzGerald, Ira Pastan, Robert J Kreitman, and Harry W Findley, *Emory Univ, Atlanta, GA, and Nci/Nih, Bethesda, MD*

The disulfide-stabilized recombinant immunotoxin RFB4(dsFv)-PE38 (BL22) contains the variable domains of the anti-CD22 monoclonal antibody RFB4 fused to a truncated *Pseudomonas* exotoxin (PE38) via an engineered disulfide bond. BL22 was recently reported to induce complete regressions of human CD22 positive lymphoma in mice, and is curently undergoing phase I testing in adults with refractory B-cell lymphoma and chronic lymphocytic leukemia (Kreitman et al, Int J Cancer 81:148, 1999). To test its efficacy in treating pediatric patients with CD22 positive BCP-ALL, BL22 was tested against the BCP-ALL cell line EU-1, cells from 10 children with BCP-ALL, and 1 with T-cell ALL. Both a 72-hr protein synthesis inhibition assay (^{3}H-leucine incorporation) and a colorimetric viability assay (WST-1) were used. The IC_{50} (50% inhibitory concentration) for EU-1 was 2.3 ng/ml by ^{3}H-leucine incorporation, and 25 ng/ml by WST-1. BCP-ALL patient cells could be categorized (by ^{3}H-leucine incorporation)as highly sensitive (IC_{50} <10 ng/ml, n=6; median=3 ng/ml), moderately sensitive (IC_{50} = 10-50 ng/ml, n=3; IC_{50} = 10, 22, 43), or relatively resistant (IC_{50} >50 ng/ml, n=1; IC_{50}=88). IC_{50} values were somewhat higher by the WST-1 assay, although results from both assays correlated well. BL22 showed no activity against T-ALL cells. Sensitivity (IC_{50}) was inversely correlated (r= -0.7) with the level of CD22 expression, calculated as the product of %CD22 positive cells and mean intesity of fluorescense (MESF) value. These data suggest that BL22 is active against leukemic cells from the majority of pediatric patients with CD22 positive BCP-ALL, and may be useful in treating this disease.

#1835 A NOVEL EUKARYOTIC VECTOR SYSTEM FOR THE RAPID CONSTRUCTION AND EVALUATION OF TETRAVALENT AND BISPECIFIC RECOMBINANT ANTIBODIES. Wijnand Helfrich, Renske Hoeven, and Lou F M H Leij, *GUIDE at Univ Hosp Groningen, Groningen, Netherlands*

We constructed a versatile eukaryotic expression vector in which two scFv antibody fragments can be inserted in tandem and fused to the Fc domain derived from human IgG1. An important feature of this vector is the presence of two multiple cloning sites (MCS) separated by an in frame linker sequence. The first MCS was specifically designed to contain unique SfiI and NotI restriction enzyme sites that can be used for directional and in frame insertion of scFv s (or potentially any molecule) selected from established phage-display systems. The second MCS can accommodate a scFv after PCR manipulation. Using this new vector, a large series of functional tetravalent and bispecific IgG-like antibodies was constructed, including tetravalent bispecific IgG-like antibodies with dual anti-tumor specificity (anti-CEA and anti-EGP2/GA733-2), and with bivalent bispecificity for retargeted cellular cytotoxicity towards human carcinoma cells. Supernatants derived from transfected COS-7 cells, were directly assayed for the biological and/or targeting activity of the excreted fusion proteins without any prior purification steps and thus in the presence of serum (10%). This procedure identified fusion proteins that have favourable characteristics like stability and biological activity in the presence of serum and at low protein concentrations.

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